Impact of Phyto-geographical variation on Phytochemical(s) content and antioxidant activity in *Ocimum sanctum* Linn. collected from different locations of West Bengal

Ph.D. Synopsis

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INTRODUCTION

- Tulsi or Holy Basil (*Ocimum sanctum* Linn) is widely distributed in India from the sea level to up to 1800 meters altitude in the Himalayas (Wealth of India, 1991).
- Its medicinal properties have been described in the Indian medicinal text Ayurveda (The science of Life) which is believed to be about 5000 years old.
- Traditionally, various parts of this plant have been used for different ailments such as cough and cold, asthma, bronchitis, digestive disorders, skin problems, eye and ear infections, undifferentiated fever, snake and scorpion bites (Ghosh, 1995).
- Scientific explorations of traditional medicinal claims of Tulsi got the momentum in the middle part of the 20th century. Most of the scientific evidences of medicinal properties of this plant were observed largely on experimental animal studies with only a few human studies.
- These studies have shown notable properties such as antimicrobial, adaptogenic, anti-diabetic, hepato-protective, anti-inflammatory, anti-carcinogenic, radio-protective, neuro-protective, cardio-protective and larvicidal / mosquito repellent of different parts of the Tulsi plant (Mondal et al, 2009).
- Experimental animal studies have clearly shown immuno-modulatory properties in the extract of Tulsi leaves (Godhwani et al, 1988; Singh et al, 1995; Mediratta et al, 2002; Mukherjee et al, 2005).
- It is often believed in India that taking Tulsi leaves on empty stomach is beneficial and improves immunity, thus the present study was designed to determine the immuno-modulatory properties of Tulsi leaf ethanolic (70%) extract in healthy volunteers through a double-blind randomized controlled trial.

Health Benefits Today

- Vitamin A is essential for vision, healthy mucus membranes and skin.

- Basil is at the top of the list for foods with Vitamin K, which plays a role in blood clotting and bone strengthening. Supports nervous and immune systems. Keeps blood sugar stable.
- Animal studies indicate basil stimulates the immune system by increasing production of disease fighting antibodies by 20%.
- Basil is listed on the FDA's GRAS (Generally Recognized as Safe) herb list.

Major Bio-active Compounds found in Ocimum:

Compound(s)	Fresh Sample (% Mean)	Dry Sample (% Mean)
Eugenol	57.36	11.23
B-Elemene	7.82	10.9
E-Caryophyllene	15.6	18.12
Germacrene	9.2	3.6
Selinene	0.6	5.3
Caryophyllene oxide	3.4	40.26

REASONS BEHIND TO SELECT THE THEME

Chemical composition of aromatic medicinal plants varies with season to season and place of cultivation. Tulsi plant grows almost in every region of India, which has varying geographical and environmental conditions in different regions. The present study is designed to compare the Phytochemical constituents of crud drug extract(s) obtained from different locations of West Bengal for their dry as well as fresh plant parts.

Figures: *Ocimum sanctum*: Holy Basil: Tulsi, Family: Lamiaceae

Why this plant…..…….?

- Ayurvedic Uses
- Antioxidant
- Antifungal
- Antiinflammatory
- Hypolipidaemic
- Antibacterial
- Leprosy Traditional uses
- Liver disease
- Body pain
- Fever
- Inflammation
- In treatment of skin diseases
- Mental Disorder
- Immunity Booster

Ayurvedic Products

- **Tulsi Ark**
- **Panch Tulsi**
- **Tulsiu haritki vati**
- **Tulsi-giloy vati, etc**

Estimated Annual Trade (Tones)

- **5000 - 10000**
- **Annual Growth Rate (%):** 10
- **Price Range (Rs. per Kg) :** 45–55

Source: NMPB-2012

METHODOLOGY

Studying the impact of phyto-geographical variation on phytochemical content and antioxidant activity in a medicinal plant like *Ocimum sanctum* (Holy Basil) collected from different locations in West Bengal involves a comprehensive methodology that combines botanical, chemical, and analytical techniques. Here's an outline of the methodology that could be applied:

Select multiple collection sites across different geographical regions in West Bengal. Ensure that the sites are diverse in terms of climate, soil type, altitude, and other relevant factors. Collect plant samples during the appropriate season and at the same stage of growth to minimize variations.

Plant Identification and Authentication:

Accurately identify and authenticate the collected plant specimens using botanical taxonomic keys. Proper identification is crucial to ensure that the correct species (*Ocimum sanctum*) is being studied.

Sample Preparation:

Prepare the collected plant samples by cleaning, drying, and grinding them into a uniform powder. Make sure to remove any extraneous materials that could interfere with the analysis.

Phytochemical Extraction:

Used hot Soxhlet extraction technique to isolate phytochemical compounds from the powdered plant material. Different solvents (e.g., ethanol, methanol, water) can be employed for different classes of compounds. Perform extractions in triplicate for each sample to ensure reproducibility.

Phytochemical Analysis:

Employed various analytical methods to quantify the phytochemical compounds present in the extracts. High-Performance Thin Liquid Chromatography (HPTLC), Gas Chromatography- Mass Spectrophotometry (GC-MS) and Spectrophotometric assays (for specific compounds like phenolics, flavonoids, alkaloids) will be used.

Antioxidant Activity Assessment:

Measure the antioxidant activity of the plant extracts using methods such as the DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging assay, ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) assay, and Ferric Reducing Antioxidant Power (FRAP) assay. These assays help determine the ability of the extracts to neutralize free radicals.

OBJECTIVE

To achieve the target of this study, objectives will be concluded within:

1) Survey and selection of ethno-botanically valuable accessions of selected plant.
2) Collection and development of passport data for the plant of target.
3) Physico-chemical estimations and In vitro evaluation of antioxidant potentiality of crude extract.
4) Quantitative estimation of selected secondary metabolites, enzymatic & non-enzymatic antioxidants.

Objectives vis a vis activities

Objective-1	**Activities**
1) Survey and selection of ethno-botanically valuable accessions of selected plant. 2) Collection and development of passport data for the plant of target. 3) Physico-chemical estimations and In vitro evaluation of antioxidant potentiality of crude extract. 4) Quantitative estimation of selected secondary metabolites, enzymatic & non-enzymatic antioxidants.	1. Survey & Collection of *Ocimum sanctum* from different phyto-geographical zones of West Bengal. 2. Preservation and Processing as per Good Lab Practice 3. Passport Data Preparation as per the details of the plant and their location. 4. All the investigations related to the physio-chemical standardization of the selected plant like, water soluble extractive, alcohol soluble extractive, total ash value, acid insoluble ash value etc. will be done according to the methods describes in the Ayurvedic Pharmacopeia of India (API) / phyto-pharma mode. 5. The samples will be assayed for their various chemical constituents (nutritional and anti-nutritional substance) e.g., micronutrients, phenol, flavonoids, Terpenoids, Alkaloids, starch, sugar, tannin etc. as Ayurvedic Pharmacopeia of India (API). In vitro Enzymatic Antioxidant Potential Activity Test: Catalase, Ascorbate Peroxidase, GR, DPPH, FRSA, Beta-Carotene Bleaching Assay.

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